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Effect of Different Foods on Body Condition and Cellular Immunity in Siberian Hamsters

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Abstract

Food quality is one of the most important factors influencing animals' immunity. In the present study, Siberian hamsters (*Phodopus sungorus*) were used to understand the effect of feeding purple cabbage on body condition and cellular immunity. Male hamsters were randomly assigned to the normal food group (NF, n = 10), normal food plus purple cabbage group (NF + PC, n = 10), and purple cabbage group (PC, n = 10), respectively. The treatment period lasted for 28 days. We found that body mass in the PC group was lower than in the other two groups after 7 days of treatment. The masses of wet carcass, subcutaneous fat, retroperitoneal fat, mesenteric fat, perigonadal fat, and total body fat were the lowest in the PC group among the three groups, while these parameters did not differ between the NF and NF + PC groups. No differences were observed in the wet masses of the thymus and spleen among the three groups. Phytohaemagglutinin (PHA) response, indicative of cellular immunity after 6 h, 12 h, 24 h, and 48 h of PHA injection, did not differ among the NF, NF + PC, and PC groups, indicating that feeding purple cabbage had no effect on cellular immunity. In summary, feeding purple cabbage decreased body mass and different parts of body fat, but did not influence cellular immunity.

Keywords

Body Fat, Phytohaemagglutinin Response, Purple Cabbage, Siberian Hamsters (*Phodopus sungorus*)

1. Introduction

Animals' immune system plays an important role in fighting against environmental pathogens (*i.e.*, viruses, bacteria, and fungi) and hence is crucial for their survival [1]. However, immune function is affected by many environmental factors such as

season [2]-[5], photoperiod [6] [7], temperature [8] [9], food [10] [11], and so on. Food quantity and quality are important factors influencing immune responses [12] [13].

Phytohaemagglutinin (PHA) response has been used to assess cellular immunity in birds and small mammals, which is generally responsible for controlling intracellular pathogens [10] [14]. Immune organs such as the thymus and spleen are also indirect immunological parameters, in which the thymus is crucial for primary T cell development and a larger spleen indicates stronger immune responses [12] [15]. Adipose tissues are not only passive energy depots, but have also been regarded as important endocrine and immune organs [16] [17].

Some researchers have investigated the effect of food quality on immune function in animals. For instance, high-protein food had no effect on the wet masses of immune organs (*i.e.*, thymus and spleen) and humoral immunity in Mongolian gerbils (*Meriones unguiculatus*) [18]. Feeding carrots could enhance cellular immunity in Kunming mice [19]. Similarly, feeding peanuts could also increase cellular immunity in striped hamsters (*Cricetulus barabensis*) [20]. In the present study, we want to know how purple cabbage would affect immune function in Siberian hamsters (*Phodopus sungorus*). Purple cabbage (*Brassica oleracea* var. *Capitata* F. *rubra*) is one of the world's most widely consumed vegetables with high nutritional values, containing the antioxidants and anti-inflammatory activity of anthocyanins, vitamins, and minerals [21] [22]. We hypothesized that feeding purple cabbage would affect body condition and immune function, and predicted that feeding purple cabbage alone would reduce energy reserves but increase cellular immunity in hamsters.

2. Materials and Methods

2.1. Animals and Experimental Design

All animal procedures were approved by the Animal Protection and Utilization Committee of Qufu Normal University. Male Siberian hamsters aged about 6 weeks were bought from the pet market in Zou city and were transported to our animal house in Qufu Normal University. The housing condition was under a constant photoperiod of 12L:12D (12 h:12 h light-dark cycle) and a temperature of 23˚C ± 1˚C. Hamsters had free access to standard rat pellet chow (Beijing KeAo Feed Co., Beijing, China) and water in plastic cages (30 cm × 15 cm × 20 cm) with sawdust as bedding. The macronutrients of the diet were 6.2% crude fat, 18% crude protein, 23.1% neutral fiber, 5% crude fiber, 12.5% acid detergent fiber, and 10.0% ash, and the caloric value was 17.5 kJ/g. After about one week of adaptation, thirty males were selected and randomly assigned into the normal food group (NF, n = 10), normal food plus purple cabbage group (NF + PC, n = 10), and purple cabbage group (PC, n = 10), respectively. Five hamsters were housed in one plastic cage (30 cm × 15 cm × 20 cm). The food in the NF group was standard rat pellet chow (Beijing KeAo Feed Co., Beijing, China). The food in the NF + PC group was provided with standard rat pellet chow and fresh purple cabbage bought from

Baiyi supermarket in Qufu Normal University. The food in the PC group was provided with only fresh purple cabbage. Food and bedding sawdust were replaced at 9:00 am every 3 days to minimize the effect of the circadian rhythm, and at the same time, body mass was measured. Each hamster in one cage was marked by shearing the fur of different body parts. The experimental time lasted for 28 days. Day 0 and day n represented the initial day and n days after experimental treatment, respectively. One hamster in the PC group died after 16-day treatment, and one hamster in the NP + PC group was found to be female after dissection at the end of the experiment. Therefore, these two hamsters were not included in the subsequent statistical analysis.

2.2. Body Composition Assay

Body composition was measured according to our previous research [8]. In brief, immune organs, including the thymus and spleen, were dissected and weighed (±1 mg). All the other visceral organs were removed to obtain a wet carcass. Moreover, subcutaneous fat, retroperitoneal fat, mesenteric fat, and perigonadal fat were carefully dissected and weighed. The sum of the four measures of subcutaneous, retroperitoneal, mesenteric, and perigonadal fat mass was regarded as the total body fat mass. The percent content of subcutaneous fat, retroperitoneal fat, mesenteric fat, perigonadal fat, and total body fat mass was determined by dividing each respective fat mass by the mass of wet carcass [8].

2.3. Cellular Immunity Assay

PHA response (*i.e.*, cellular immunity) was measured as in previous studies [10] [15]. Specifically, hamsters in the NF, NF + PC, and PC groups on day 24 were caught, and we measured the footpad thickness of the left hind foot with a micrometer (Digimatic Indicator ID-C Mitutoyo Absolute cod. 547-301, Japan) to ± 0.01 mm. Immediately thereafter, hamsters in the three groups were injected subcutaneously with 0.1 mg of PHA (PHA-P) dissolved in 0.03 mL of sterile saline (pH 7.4) in the middle of the footpad. After 6 h, 12 h, 24 h, and 48 h of injection, we measured the footpad thickness. The PHA response was calculated as the difference between pre- and post-injection measurements divided by the initial footpad thickness (PHA response = (post PHA – pre PHA)/pre PHA). Six measurements of footpad thickness were taken at each of the 4 time points to obtain the value of each hamster [10].

2.4. Statistical Analysis

Data were analyzed using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). The changes in body mass and PHA responses with treatment time, as well as the interaction of treatment time and groups, were analyzed using the Repeated Measures of General Linear Model multivariate analysis. The differences in body mass and PHA responses among the NF, NF + PC, and PC groups at certain time points were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's *post hoc*

tests. Group differences in body composition were also analyzed by one-way ANOVA followed by Tukey's post hoc tests. Group differences in thymus and spleen mass, with final body mass as the covariate, were analyzed by General Linear Model multivariate analysis followed by Bonferroni post hoc tests. Pearson correlation analysis was performed to determine the correlations of PHA responses with final body mass and total body fat mass for all hamsters. Results are presented as mean ± s.e.m., and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Body Mass

Body masses changed significantly with treatment time ($F_{9,225} = 20.792$, $P < 0.001$), and were affected significantly by the interaction of treatment time and groups ($F_{18,225} = 5.616$, $P < 0.001$). Specifically, body masses increased in the NF group ($F_{9,81} = 10.233$, $P < 0.001$) and the NF + PC group ($F_{9,81} = 10.233$, $P < 0.001$), respectively. However, body mass in the PC group remained stable with treatment time ($F_{9,72} = 1.174$, $P = 0.325$) (Figure 1). Moreover, there were no significant differences in body mass among the NF, NF + PC and PC groups from Day 1 ($F_{2,27} = 0.145$, $P = 0.866$) to Day 4 ($F_{2,27} = 2.566$, $P = 0.096$). However, body mass differed significantly among the three groups on Day 7 ($F_{2,27} = 5.032$, $P = 0.015$), Day 10 ($F_{2,27} = 4.401$, $P = 0.023$), Day 13 ($F_{2,27} = 8.167$, $P = 0.002$), Day 16 ($F_{2,27} = 10.489$, $P < 0.001$), Day 19 ($F_{2,27} = 8.412$, $P = 0.002$), Day 22 ($F_{2,27} = 8.349$, $P = 0.002$), Day 25 ($F_{2,27} = 7.677$, $P = 0.003$), and Day 28 ($F_{2,26} = 8.803$, $P = 0.001$) (Figure 1).

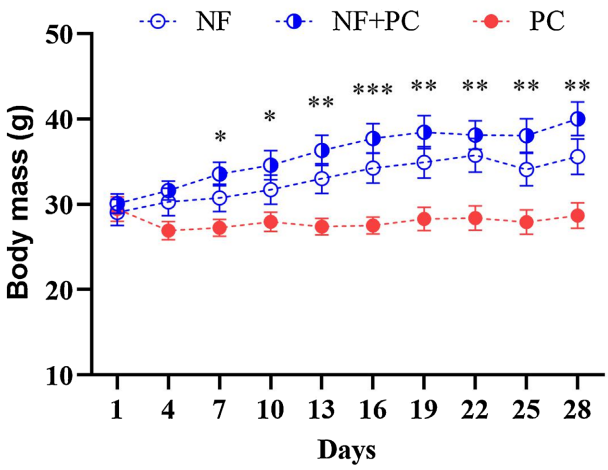

Figure 1. Influence of different food on body mass in male Siberian hamsters. NF: Normal food; NF + PC: Normal food plus purple cabbage; PC: Purple cabbage. One star (*) indicates $P < 0.05$, two stars (**) indicate $P < 0.01$ and three stars (***) indicate $P < 0.001$ among the three groups at a certain time, respectively.

3.2. Body Composition

Final body mass, the masses of wet carcass, subcutaneous fat, retroperitoneal fat, mesenteric fat, perigonadal fat, and total body fat were the lowest in the PC group among the three groups, while these parameters did not differ between the NF and

NF + PC groups (Table 1). Moreover, the percentages of retroperitoneal fat mass, perigonadal fat mass, and total body fat mass were also the lowest in the PC group among the three groups, whereas these indices did not differ between the NF and NF + PC groups (Table 1). The percentages of subcutaneous fat mass, retroperitoneal fat mass, and mesenteric fat did not differ among the three groups (Table 1).

Table 1. Influence of different foods on body composition in male Siberian hamsters.

Parameters	NF	NF + PC	PC	Statistical summary	
Sample size	10	9	9	$F_{2,27}$	P
Initial body mass (g)	29.0 ± 1.5	30.1 ± 1.2	29.5 ± 1.5	0.145	0.866
Final body mass (g)	35.6 ± 2.1^{a}	40.0 ± 2.0^{a}	28.7 ± 1.5^{b}	8.803	0.001
Wet carcass mass (g)	23.8 ± 1.6^{a}	27.2 ± 1.8^{a}	16.9 ± 1.0^{b}	11.676	<0.001
Subcutaneous fat (g)	0.938 ± 0.280	0.971 ± 0.226	0.220 ± 0.056	3.739	0.038
Subcutaneous fat content (%)	3.697 ± 1.076	3.404 ± 0.654	1.234 ± 0.322	2.929	0.072
Retroperitoneal fat (g)	0.249 ± 0.060^{ab}	0.370 ± 0.135^{a}	0.060 ± 0.017^{b}	3.316	0.053
Retroperitoneal fat content (%)	1.029 ± 0.254	1.193 ± 0.350	0.320 ± 0.091	3.206	0.058
Mesenteric fat (g)	0.368 ± 0.060^{a}	0.425 ± 0.033^{a}	0.204 ± 0.026^{b}	6.633	0.005
Mesenteric fat content (%)	1.506 ± 0.161^{a}	1.556 ± 0.019^{a}	1.230 ± 0.163^{b}	1.570	0.228
Perigonadal fat (g)	0.816 ± 0.102^{a}	0.931 ± 0.128^{a}	0.210 ± 0.054^{b}	13.200	<0.001
Perigonadal fat content (%)	3.322 ± 0.279^{a}	3.323 ± 0.296^{a}	1.014 ± 0.262^{b}	22.278	<0.001
Total body fat (g)	2.486 ± 0.432^{a}	2.859 ± 0.462^{a}	0.735 ± 0.121^{b}	8.789	0.001
Total body fat content (%)	10.037 ± 1.393^{a}	10.066 ± 1.054^{a}	4.128 ± 0.537^{b}	9.546	0.001

Data are mean ± SE. Values for a specific parameter that share different superscripts are significantly different at $P < 0.05$, determined by one-way ANOVA and Turkey's post-hoc tests.

3.3. Immune Organs

No difference was observed in the wet masses of the thymus ($F_{2,27} = 1.478$, $P = 0.249$) and spleen ($F_{2,27} = 2.364$, $P = 0.117$) among the NF, NF + PC, and PC groups (Figure 2(a) & Figure 2(b)).

3.4. Cellular Immunity

To describe simply, PHA 6 h, 12 h, 24 h, and 48 h indicated that PHA responses after 6 h, 12 h, 24 h, and 48 h of PHA injection, respectively. PHA response decreased significantly with treatment time ($F_{3,78} = 96.526$, $P < 0.001$), but was not impacted by the interaction of treatment time and groups ($F_{6,78} = 1.469$, $P = 0.201$).

Specifically, PHA response in the NF ($F_{3,27}$ = 33.962, P < 0.001), NF + PC ($F_{3,27}$ = 22.533, P < 0.001) and PC ($F_{3,27}$ = 48.349, P < 0.001) groups all reduced with treatment time, respectively (Figure 3). No significant differences were observed in PHA 6 h ($F_{2,27}$ = 0.565, P = 0.575), 12 h ($F_{2,27}$ = 0.307, P = 0.739), 24 h ($F_{2,27}$ = 1.830, P = 0.181), and 48 h ($F_{2,27}$ = 2.715, P = 0.086) (Figure 3). Final body mass was not correlated with PHA 6 h (r = −0.054, P = 0.783), PHA 12 h (r = −0.007, P = 0.973), PHA 24 h (r = 0.232, P = 0.234), PHA 48 h (r = 0.041, P = 0.835). No correlations were observed between total body fat mass and PHA 6 h (r = −0.066, P = 0.738), PHA 12 h (r = 0.022, P = 0.912), PHA 24 h (r = 0.248, P = 0.202), and PHA 48 h (r = 0.059, P = 0.766), respectively.

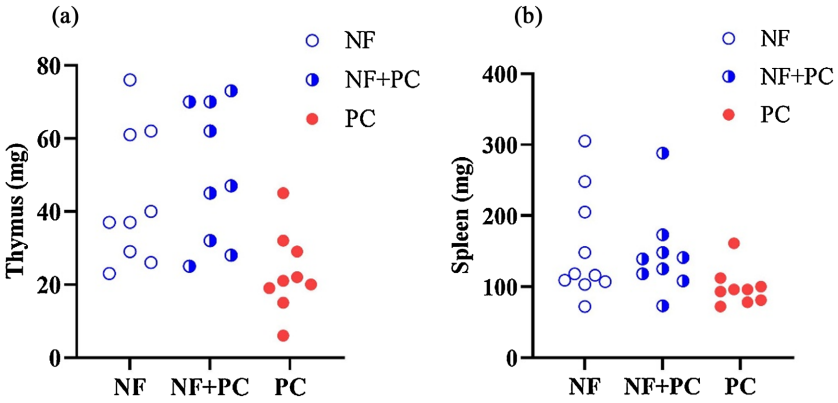

Figure 2. Influence of different food on the wet masses of thymus (a) and spleen (b) in male Siberian hamsters. NF: Normal food; NF + PC: Normal food plus purple cabbage; PC: Purple cabbage.

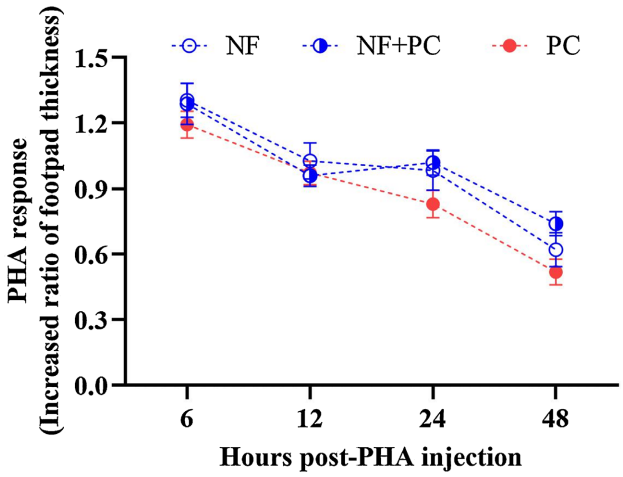

Figure 3. Influence of different foods on PHA response in male Siberian hamsters.

4. Discussion

As expected, feeding purple cabbage alone decreased body mass in the PC group compared with the NF and NF + PC groups. This result may be due to the lower energy content of purple cabbage compared with the normal food, although it has high nutritional values, containing the antioxidants and anti-inflammatory activ-

ity of anthocyanins, vitamins, and minerals [23]. Therefore, after 28 days of feeding purple cabbage, body mass in the PC group was 19.4% lower, while body mass in the NF + PC group was 12.3% higher than that of the NF group, respectively. This result indicates that high-energy food plays an important role in maintaining or increasing body mass. In addition, the normal food plus purple cabbage (NF + PC) group had the highest final body mass, even if not statistically different from the normal food group. This result implied that dietary supplementation of purple cabbage had a boosting effect on body mass growth. Moreover, the masses of wet carcass, subcutaneous fat, retroperitoneal fat, mesenteric fat, perigonadal fat, and total body fat were also reduced in the PC group compared with the other two groups. These findings demonstrated that hamsters mobilized energy reserves, including subcutaneous fat, retroperitoneal fat, mesenteric fat, and perigonadal fat, to meet the lower energy food (*i.e.*, purple cabbage).

Contrary to our expectation, feeding purple cabbage alone did not affect cellular immunity and the wet masses of the thymus and spleen in Siberian hamsters. It is thought that mounting or maintaining immune responses is costly in terms of energy [24] [25]. Energy depots (*i.e.*, white adipose tissues) may provide energy for many physiological processes, including immune responses; consequently, they play an important role in mounting or maintaining immune responses [24] [26]. In general, animals with lower energy reserves allocate less energy to immune responses than those with higher reserves, and hence animals with lower energy reserves would have weaker immune responses [27] [28]. For instance, reduction in body fat masses caused by starvation led to the suppression of immunity in mice [29]. In our study, feeding purple cabbage alone had no influence on cellular immunity and the wet masses of the thymus and spleen. The reason might lie in the fact that purple cabbage has high nutritional value, containing the antioxidants and anti-inflammatory activity of anthocyanins, vitamins, and minerals, which might offset the suppressive effect of reduced body fat mass [21] [22]. Our previous studies have shown that feeding carrots or peanuts could enhance cellular immunity in Kunming mice or striped hamsters [19] [20]. It appears that different functional foods have distinct impacts on animal physiology.

The present study also had some limitations. First, the hamsters were housed in groups (*i.e.*, 4 - 5 hamsters/cage). Therefore, all the detected parameters might be affected by housing density. Second, this study did not measure the food intake or energy intake of the three groups. Consequently, there were no data on the quantity of food consumed by each group, which would have provided more direct evidence for differences in energy intake. Lastly, the sample size of each group was relatively small.

In summary, feeding purple cabbage alone decreased body mass and the masses of wet carcass, subcutaneous fat, retroperitoneal fat, mesenteric fat, perigonadal fat, and total body fat, indicating that hamsters mobilized energy reserves to meet the lower energy food. However, feeding purple cabbage alone had no effect on cellular immunity and the masses of the thymus and spleen, implying no influence

on immune function in hamsters. The role of the nutritional components of purple cabbage on immune function needs further research in the future. Our findings also had theoretical or practical implications. For example, the results may help us to understand the foraging strategy of trade-offs in wild animals or how to care for animals.

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Authors' Contributions

D.L.X. designed the study and supervised the analyses. Y.F.T. wrote the draft paper. S.C.Z. and C.Y.Y. performed the experiment. D.L.X. revised the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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